Applicant(s): David A. Sanders et al.

Serial No. 09/762,224

Int'l Filing Date: 4 August 1999

For: PSEUDOTYPED RETROVIRUSES AND STABLE CELL LINES FOR THEIR PRODUCTION

preferably obtained from the Moloney murine leukemia virus (MMLV; in the genus Oncovirus C). Such sequences are well known in the art. For example, nucleotide sequences encoding MMLV gag, pro and pol may be found in Van Bereven et al. *Cell* (1981)27:97-108. Most preferably, such sequences are obtained from lentiviruses. Unlike most retroviruses, lentiviruses have the capacity to integrate the genetic material they carry into the chromosomes of non-dividing cells as well as dividing cells. Therefore, lentiviral nucleotide sequences encoding proteins that allow for chromosomal integration of virally transported nucleic acid in non-dividing cells are advantageously employed, as the host range of the peudotyped retroviruses will be broadened.

Please replace the paragraph beginning at page 13, line 11, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

In one form of the present invention, the cells include nucleotide sequences encoding glycoproteins from an alphavirus. In a most preferred embodiment, the cells include nucleotide sequences encoding glycoproteins from the viral species Ross River (depicted in SEQ ID NO:1 and SEQ ID NO:2). The viral transmembrane glycoprotein complex that is responsible for the binding of the alphavirus to the surface of a susceptible cell and for the fusion of the viral and cellular membranes that occurs during the process of viral entry includes a trimer of a heterodimer of two transmembrane proteins, which are denoted  $E_1$  and  $E_2$  and which are encoded by an  $E_3$ - $E_2$ -6K- $E_1$  glycoprotein coding region ( $E_3$  and 6K refer to viral proteins involved in maturation of  $E_1$  and  $E_2$  as known in the art) on the alphaviral genome. The  $E_2$ - $E_1$  coding region includes an  $E_3$  glycoprotein coding region as well as the 6K protein coding region. Such nucleotide sequences may be obtained by methods known to the skilled artisan as discussed for the gag, pro and pol nucleotide sequences above. For example, the  $E_2$ - $E_1$  coding region may be obtained as discussed in Kuhn et al. (1991) Virology 182:430-441. The  $E_2$ - $E_1$  glycoprotein coding region is also operably linked to a promoter sequences, such as described above, at its 5' end.

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Please replace the paragraph beginning at page 14, line 9, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

In another embodiment, the cells include nucleotide sequences encoding glycoproteins from a filovirus. Such filoviruses also exhibit a broad host range. A wide variety of nucleotide sequences that encode filoviral glycoproteins may be used to produce the inventive cells of the present invention. For example, nucleotide sequences encoding glycoproteins from the Marburg and Ebola virus (in the family Filoviridae and, including, for example, Ebola-Zaire and Ebola-Reston) may be introduced into the cells described above to produce a pseudotyped retrovirus. SEQ ID NO:3 shows the Ebola Zaire glycoprotein-encoding sequence and SEQ ID NO:5 shows the Marburg virus glycoprotein-encoding sequence. The nucleotide sequences encoding the filoviral glycoproteins may be obtained as described in Sanchez et al. (1993) *Virus Res.* 29(3):215-240 and Will et al., (1993) *J. Virol.* 67:1203-1210. Moreover, such sequences may be obtained by other methods known to those skilled in the art, as described above for the togaviruses.

Please replace the paragraph beginning at page 37, line 17, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

pEZGP1 was produced by cloning into the polylinker of plasmid pcDNA3 nucleotide sequences corresponding to nucleotides 6029-8253 [sequences 6029-8253, corresponding to nucleotides 132-2354 described in Genbank as Accession Number U23187, are shown in SEQ ID NO:3 from the Ebola Zaire virus genome, with the exception that an additional "a" has been inserted between nucleotides 1027 and 1028 in SEQ ID NO:3 compared to the Genbank sequence] from the complete Ebola Zaire genome [described in Sanchez et al. (1993) *Virus Res.* 29(3):215-240] obtained by digestion of the MP1153 plasmid provided by Dr. Anthony Sanchez with Eco Rl and Hindlll. SEQ ID NO:4 shows the amino acid sequence of the Ebola Zaire glycoprotein.